Extraction and Purification of the Brucella Abortus Spp. RB51 OMP's by Sodium Deoxycholate and Sodium Laurylsarcosinate Methods in Laboratory Animals

Niayesh Javadi[1]; Hojat Ahmadi*[2]; Mehdi Nejati[2]; Bahareh Rahimian Zarif[1]; Reza Saghiri[3]; Mahdi Sadati[1,2]; Sara Zahednia[2]; Marzieh Esmailzadeh[1,2]; Setareh Taher bahrami[1,2]; Fahimeh Gholizadeh[1,2]; Mahya Bayat[2]; Sanaz Shekofti[1,2]

1-Department of Microbiology, Islamic Azad University Science, Research Campus, Kurdistan Branch, Kurdistan, Iran

2- Department of Bacterial Vaccines, Antigen Production, Pastuer Institute of Iran, Tehran, Iran
3-Department of Biochemistry, Pasteur Institute of Iran, Iran

hojiajmadi@yahoo.com

Background & Objectives: In recent years, the use of of *Brucella abortus* S19, and *Brucella melitensis* rev1, and *Brucella suis* S105, and S2, and *Brucella abortus* 4520 live vaccines, and *Brucella melitensis* H38 killed vaccines were common for brucellosis control. However, the vaccination of brucellosis occures in the first six months which the livestock are not yet pregnant and the second six month is not appropriate, which it causes abortion. Therefore, vaccines should be developed with high efficacy and low side effects. Regarding these issues, the Outer Membrane Proteins (OMP's) of *Brucella ssp*. could be considered as an immunostimulator molecule. Our aim was to extract and purify the Outer Membrane Proteins of Brucella abortus, by two methods: Sodium Deoxycholate and Sodium Lauroylsarcosinate.

Methods: The OMP's of this bacterium were extracted by two methods: the Sodium Deoxycholate and the sodium Laurylsarcosinate methods of extraction. Two groups of white New Zealand Rabbits (n=10) were selected for immunization, each rabbit was subcutaneously inoculated with 0.5 mL serum associated with incomplete Freund's adjuvant and serum physiology including 50µg of the OMP's, twice at a 15 day interval. The heart blood was drawn 15 days after the last inoculation and its serum was separated by centrifugation and the bactericidal titer of the antibody was evaluated by the Serum Bactericidal Assay Methods.

Results: In the Deoxycholate methods of extraction, the cellular immunity is not stimulated. The purified OMP's extracted in this study has a low content of Nucleic acid and LPS. Compared to deoxycholate methods, in sodium lauroylsarcosinate methods, the cell wall was broken by ultrasound and as a result, the LPS would run off. Therefore the OMP portion containes nucleic acid and LPS. The nucleic acid and LPS content is higher than the sodium lauroylsarcosinate methods.

Keywords: Outer Membrane Proteins; *Brucella abortus* Vaccine; Sodium Lauroylsarcosinate; Sodium Deoxycholate